Chapter 9

Measles

MEASLES IS AN ACUTE VIRAL INFECTIOUS DISEASE. THERE ARE references to measles as far back as the 7th century A.D. The disease was described by Rhazes in the 10th Century A.D. as "more dreaded than smallpox."

In 1846, Peter Panum described the incubation period of measles and lifelong immunity. Enders and Peebles isolated the virus in human and monkey kidney tissue culture in 1954. The first live attenuated vaccine was licensed for use in the U.S. in 1963 (Edmonston B strain).

Measles
- **Highly contagious viral illness**
- **First described in 7th century**
- **Near universal infection of childhood in prevaccination era**
- **Frequent and often fatal in developing areas**

Measles Virus

The measles virus is a paramyxovirus, genus Morbillivirus. It is 100 to 200nm in diameter, with a core of single-stranded RNA, and is closely related to the rinderpest and canine distemper viruses. Measles virus has six structural proteins, of which three are complexed to the RNA and three are associated with the viral membrane envelope. Two of the membrane envelope proteins are most important in pathogenesis. They are the F (fusion) protein, which is responsible for fusion of virus and host cell membranes, viral penetration, and hemolysis, and the H (hemagglutinin) protein which is responsible for adsorption of virus to cells.

Measles Virus
- **Paramyxovirus**
- **One antigenic type**
- **Recent variation in hemagglutinin glycoprotein identified**
- **Rapidly inactivated by heat and light**

There is only one antigenic type of measles virus. Although recent studies have documented changes in the H glycoprotein, these changes do not appear to be epidemiologically important (*i.e.*, no change in vaccine efficacy has been observed).

Measles virus is rapidly inactivated by heat, light, acidic pH, ether, and trypsin. It has a short survival time (<2 hours) in the air, or on objects and surfaces.

Pathogenesis

Measles
Pathogenesis

- **Respiratory transmission of virus**
- **Replication in nasopharynx and regional lymph nodes**
- **Primary viremia 2-3 days after exposure**
- **Secondary viremia 5-7 days after exposure with spread to tissues**

Measles is a systemic infection. The primary site of infection is the respiratory epithelium of the nasopharynx. Two to three days after invasion and replication in the respiratory epithelium and regional lymph nodes, a primary viremia occurs with subsequent infection of the reticuloendothelial system. Following further viral replication in regional and distal reticuloendothelial sites, there is a second viremia, which occurs 5 to 7 days after initial infection. During this viremia, there may be infection of the respiratory tract and other organs. Measles virus is shed from the nasopharynx beginning with the prodrome until 3-4 days after rash onset.

Clinical Features

The **incubation period** of measles, from exposure to prodrome averages 10-12 days. From exposure to rash onset averages 14 days (range, 7-18 days).

The **prodrome** lasts 2-4 days (range 1-7 days). It is characterized by fever, which increases in stepwise fashion, often peaking as high as 103°-105°F. This is followed by the onset of cough, coryza (runny nose), and/or conjunctivitis.

Measles
Clinical Features
Prodrome

- **Incubation period 10-12 days**
- **Stepwise increase in fever to 103 F or higher**
- **Cough, coryza, conjunctivitis**
- **Koplik spots**

Koplik's spots, an exanthem present on mucous membranes, is considered to be pathognomonic for measles. It occurs 1-2 days before the rash to 1-2 days after the rash, and appears as punctate blue-white spots on the bright red background of the buccal mucosa.

The measles **rash** is a maculopapular eruption that usually lasts 5-6 days. It begins at the hairline, then involves the face and upper neck. Over the next 3 days, the rash gradually proceeds downward and outward, reaching the hands and feet.

Measles
Clinical Features
Rash

- **2-4 days after prodrome, 14 days after exposure**
- **Maculopapular, becomes confluent**
- **Begins on face and head**
- **Spreads to trunk, arms, legs**
- **Persists 5-6 days**
- **Fades in order of appearance**

The maculopapular lesions are generally discrete, but may become confluent, particularly on the upper body. Initially, lesions blanch with fingertip pressure. By 3-4 days, most do not blanch with pressure. Fine desquamation occurs over more severely involved areas. The rash fades in the same order that it appears, from head to extremities.

Other symptoms of measles include anorexia, diarrhea, especially in infants, and generalized lymphadenopathy.

Complications

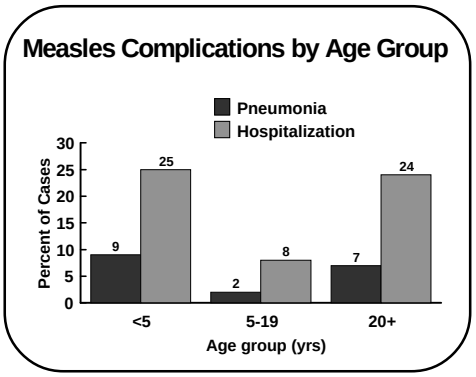
Approximately 30% of reported measles cases have one or more complications. Complications of measles are more common among children <5 and adults >20 years of age. From 1985 through 1992, **diarrhea** was reported in 8% of reported cases, making this the most commonly reported complication of measles. **Otitis media** was reported in 7% of reported cases and occurs almost exclusively in children. **Pneumonia** (6% of reported cases) may be viral or superimposed bacterial, and is the most common cause of death.

Measles
Complications

Condition	Percent reported
Any complication*	29
Diarrhea	8
Otitis media	7
Pneumonia	6
Encephalitis	0.1
Death	0.2
Hospitalization	18

*includes hospitalization
Based on 1985-1992 surveillance data

Acute **encephalitis** is reported in approximately 0.1% of reported cases. Onset generally occurs 6 days after rash onset (range 1-15 days), and is characterized by fever, headache, vomiting, stiff neck, meningeal irritation, drowsiness, convulsions, and coma. Cerebrospinal fluid shows pleocytosis and elevated protein. Case fatality rate can approximately 15%. Some form of residual neurologic damage occurs in as many as 25%. **Seizures** (with or without fever) are reported in 0.6% to 0.7% of reported cases.



Death from measles has been reported in approximately 1-2 per 1,000 reported cases in the United States in recent years. As with other complications of measles, the risk of death is higher among young children and adults. Pneumonia accounts for about 60% of deaths. The most common causes of death are pneumonia in children and acute encephalitis in adults.

Subacute sclerosing panencephalitis (SSPE) is a rare degenerative central nervous system disease believed to be due to persistent measles virus infection of the brain. Average onset occurs 7 years after measles (range 1 month-27 years), and occurs in five to ten cases per million reported measles cases. The onset is insidious, with progressive deterioration of behavior and intellect, followed by ataxia (awkwardness), myoclonic seizures, and eventually death. SSPE has been extremely rare since the early 1980s.

Measles illness during pregnancy results in a higher risk of premature labor, spontaneous abortion, and low-birth-weight infants. Birth defects (with no definable pattern of malformation) have been reported rarely, without confirmation that measles was the cause.

Atypical measles occurs only in persons who received inactivated ("killed") measles vaccine (KMV) and are subsequently exposed to wild-type measles virus. Between 600,000 and 900,000 persons received KMV in the U.S. from 1963 to 1967. KMV sensitized the recipient to measles virus antigens without providing protection. Subsequent infection with measles virus leads to signs of hypersensitivity polyserositis. The illness is characterized by fever, pneumonia, pleural effusions, and edema.

The rash is usually maculopapular or petechial, but may have urticarial, purpuric, or vesicular components. It appears first on the wrists or ankles. Atypical measles may be prevented by revaccinating with live measles vaccine. Moderate to severe local reactions with or without fever may follow vaccination; these reactions are less severe than with infection with wild measles virus.

Modified measles occurs primarily in patients who received immune globulin (IG) as post-exposure prophylaxis and in young infants who have some residual maternal antibody. It is usually characterized by a prolonged incubation period, mild prodrome, and sparse, discrete rash of short duration. Similar mild illness has been reported among previously vaccinated persons.

Rarely reported in the United States, **hemorrhagic measles** is characterized by high fever (105°-106°F), seizures, delirium, respiratory distress, and hemorrhage into the skin and mucous membranes.

Measles in an immunocompromised person may be severe, with a prolonged course. It is reported almost exclusively in persons with T-cell deficiencies (certain leukemias, lymphomas, and Acquired Immunodeficiency Syndrome [AIDS]). It may occur without the typical rash, and a patient may shed virus for several weeks after the acute illness.

Measles in developing countries has resulted in high attack rates among children <12 months of age. Measles is more severe in malnourished children, particularly those with vitamin A deficiency. Complications include diarrhea, dehydration, stomatitis, inability to feed, and bacterial infections (skin and elsewhere). The case fatality rate may be as high as 25%. Measles is also a leading cause of blindness in African children.

Laboratory Diagnosis

Isolation of measles virus is not recommended as a routine method to diagnose measles. However, virus isolates are extremely important for molecular epidemiologic surveillance to help determine the geographic origin of the virus and the viral strains circulating in the United States.

Measles virus can be isolated from urine, nasopharyngeal aspirates, heparinized blood, or throat swabs. Specimens for virus culture should be obtained from every clinically suspected case of measles and should be shipped to the state public health laboratory or CDC, at the direction of the state health department. Clinical specimens for viral isolation should be collected at the same time as samples taken for serologic testing. Because the virus is more likely to be isolated when the specimens are collected within 3 days of rash onset, collection of specimens for virus isolation should not be delayed until serologic confirmation is obtained. Clinical specimens should be obtained within 7 days of rash onset, and should not be collected more than 10 days after rash onset. A detailed protocol for collection of specimens for viral isolation is included at the end of this chapter.

Measles
Laboratory Diagnosis

- **Isolation of measles virus from clinical specimen (e.g., nasopharynx, urine)**
- **Significant rise in measles IgG by any standard serologic assay (e.g., enzyme immunoassay, hemagglutination inhibition)**
- **Positive serologic test for measles IgM antibody**

Serologic testing, most commonly by enzyme-linked immunoassay (ELISA or EIA), is widely available and may be diagnostic if done at the appropriate time. Generally, a previously susceptible person exposed to either vaccine or wild type measles virus will first mount an IgM response and then an IgG response. The IgM response will be transient (1–2 months) and the IgG response should persist for many years. Uninfected persons should be IgM negative and will be either IgG negative or IgG positive depending upon their previous infection history.

ELISA tests for IgM antibody require only a single serum specimen and are diagnostic if positive. The preferred reference test is a capture IgM test developed by CDC. This test should be used to confirm every case of measles that is reported to have some other type of laboratory confirmation. IgM capture tests for measles are often positive on the day of rash onset. However, in the first 72 hours after rash onset, up to 20% of tests for IgM may give false-negative results. Tests that are negative in the first 72 hours after rash onset should be repeated. IgM is detectable for at least 28 days after rash onset and frequently longer.

A variety of tests for IgG antibodies to measles are available and include ELISA tests, hemagglutination inhibition, indirect fluorescent antibody tests, microneutralization, and plaque reduction neutralization. Complement fixation, while widely used in the past, is no longer recommended.

IgG testing for measles requires the demonstration of a rise in the titer of antibody against measles virus, so two serum specimens are always required. The first specimen should be drawn as soon after rash onset as possible. The second specimen should be drawn 10–30 days later. The tests for IgG antibody should be conducted on both specimens at the same time. The same type of test should be used on both specimens. The specific criteria for documenting an increase in titer depends on the test. ELISA values are not titers and increases in ELISA values do not directly correspond to four-fold or greater titer rises.

Tests for IgG antibody require two serum specimens and a confirmed diagnosis cannot be made until the second specimen is obtained. As a result, IgM tests are generally preferred.

Measles
Epidemiology

• Reservoir	Human
• Transmission	Respiratory - person to person Airborne
• Temporal pattern	Peak late winter and spring
• Communicability	Maximum 4 days before to 4 days after rash onset

Epidemiology

Occurrence

Measles occurs throughout the world.

Reservoir

Measles is a human disease. There is no known animal reservoir, and an asymptomatic carrier state has not been documented.

Transmission

Measles transmission is primarily person to person via large respiratory droplets. Airborne transmission via aerosolized droplet nuclei has been documented in closed areas (*e.g.*, office examination room) for up to 2 hours after a person with measles occupied the area.

Temporal pattern

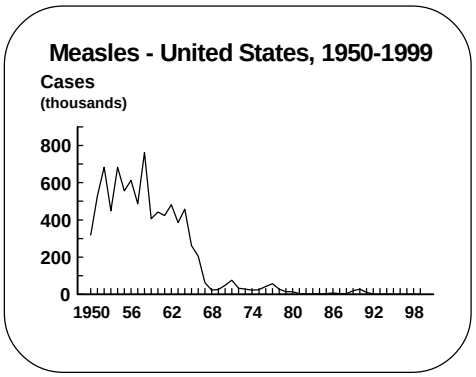
In temperate areas, measles disease occurs primarily in the late winter and spring.

Communicability

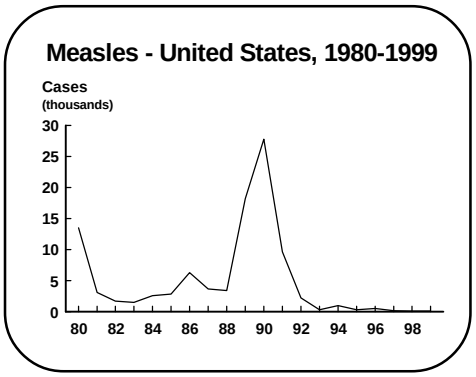
Measles is highly communicable, with >90% secondary attack rates among susceptible persons. Measles may be transmitted from 4 days prior to 4 days after rash onset. Maximum communicability occurs from onset of prodrome through the first 3-4 days of rash.

Secular Trends in the United States

Before 1963, approximately 500,000 cases and 500 deaths were reported annually with epidemic cycles every 2-3 years. However, the actual number of cases was estimated at 3-4 million annually. More than 50% of persons had measles by age 6 and more than 90% had measles by age 15. The highest incidence was in 5-9 year-olds, who generally accounted for more than 50% of reported cases.

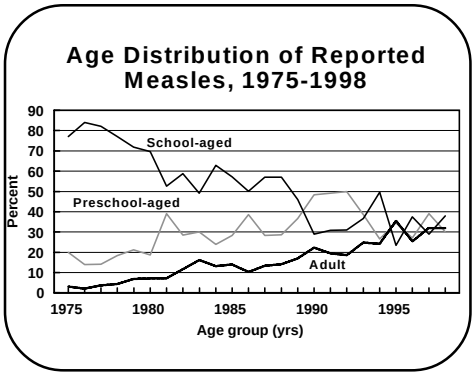


Following licensure of vaccine in 1963, the incidence of measles decreased by more than 98%, and 2-3 year epidemic cycles no longer occurred. Because of this success, a 1978 Measles Elimination Program set a goal to eliminate indigenous measles by October 1, 1982 (26,871 cases were reported in 1978). The 1982 elimination goal was not met, but in 1983, only 1,497 cases were reported (0.6 cases per 100,000 population), the lowest annual total ever reported up to that time.



The incidence of measles increased annually after 1983, to 6,282 cases (2.6/100,000 population) in 1986, and decreased slightly to approximately 3,500 cases (1.5 per 100,000 population) in 1987-1988.

During 1980-1988, a median of 57% of reported cases were among school-aged persons (5-19 years of age), and a median of 29% were among children <5 years of age. A median of 8% of cases were among infants <1 year of age.



From 1985 through 1988, 42% of cases occurred in persons who were vaccinated on or after their first birthdays. During these years, 68% of cases in school-aged children (5-19 years) had been appropriately vaccinated. The occurence of measles among previously vaccinated children led to the recommendation for a second dose in this age group.

From 1980 through 1988, a median of two measles-associated deaths per year were reported, for a median death-to-case ratio of 0.64 deaths per 1,000 reported cases.

Measles Resurgence 1989-1991
United States

• Cases	55,622
• Age group affected	Children <5 yrs
• Hospitalizations	>11,000
• Deaths	125
• Direct medical costs	>$150 million

Measles resurgence in 1989-1991

In 1989 through 1991, a dramatic increase in cases occurred. During these 3 years a total of 55,622 cases were reported (18,193 in 1989; 27,786 in 1990; 9,643 in 1991). In addition to the increased number of cases, a change in age distribution of cases occurred. Prior to the resurgence, school-aged children had accounted for the largest proportion of reported cases. During the resurgence, 45% of all reported cases were in children <5 years of age. In 1990, 48% of patients were in this age group, the first time that the proportion of cases in children <5 years of age exceeded the proportion of cases in 5-19-year-olds. Thirty-five percent of cases were among school-aged persons (5-19 years old).

Overall incidence rates were highest for Hispanics and blacks and lowest for non-Hispanic whites. Among children <5 years of age the incidence of measles among blacks and Hispanics was four to seven times higher than among non-Hispanic whites.

A total of 123 measles-associated deaths were reported (death-to-case ratio = 2.2 per 1,000 cases). Forty-nine percent of deaths were among children <5 years of age. Ninety percent of fatal cases had no history of vaccination. Sixty-four deaths were reported in 1990, the largest annual number of deaths from measles since 1971.

The most important cause of the measles resurgence of 1989-1991 was low vaccination coverage. Measles vaccine coverage was low in many cities, including some that experienced large outbreaks among preschool-aged children throughout the early to mid-1980s. Surveys in areas experiencing preschool-type measles outbreaks indicated that as few as 50% of children had been vaccinated against measles by their second birthdays, and that black and Hispanic children were less likely to be age-appropriately vaccinated than white children.

Measles susceptibility of infants less than one year of age may have increased. During the 1989-1991 measles resurgence, incidence rates for infants were more than twice as high as those in any other age group. The mothers of many infants who developed measles were young, and their measles immunity was most often due to vaccination rather than infection with wild virus. As a result, a smaller amount of antibody was transferred across the placenta to the fetus, compared with antibody transfer from mothers who had higher antibody titers resulting from wild virus infection. The lower quantity of antibody resulted in immunity that waned more rapidly, making infants susceptible at a younger age than in the past.

The increase in measles in 1989-1991 was not limited to the United States. Large outbreaks of measles were reported by many other countries of North and Central America, including Canada, El Salvador, Guatemala, Honduras, Jamaica, Mexico, and Nicaragua.

Measles in 1993-1999

Reported cases of measles declined rapidly after the 1989-1991 resurgence. This decline was due primarily to intensive efforts to vaccinate preschool-aged children. Measles vaccination levels among 2 year-old children increased from 70% in 1990 to 92% in 1998.

Since 1993, fewer than 500 cases have been reported in most years and there is no predominant age group. Available data strongly suggest that measles transmission has been interrupted. Most cases are now imported from other countries, or linked to imported cases. Most imported cases originate in Europe and Asia. Due to an aggressive measles vaccination program by the Pan American Health Organization, measles incidence is now very low in Latin America and the Caribbean. Measles elimination from the Americas appears to be an achievable goal.

Measles incidence increased to 963 cases in 1994, due primarily to several large outbreaks among persons with religious and philosophic exemption to vaccination. The 1999 provisional total of 86 cases is the lowest annual total ever reported.

Since 1994, cases among preschool-aged children have become less common, and those among school children have increased. An increased proportion of cases have occurred among adults. In 1973, persons over 20 years of age accounted for only about 3% of cases. In 1994, adults accounted for 24% of cases, and in 1998, this age group accounted for 32% of all reported cases.

Measles outbreaks

Measles outbreaks are classified into two major types based on the predominant age group affected. “Preschool” and “school-aged” outbreaks are those in which children <5 and persons 5-19 years of age, respectively, account for the greatest number of cases.

Preschool-type outbreaks involve predominantly unvaccinated children <5 years of age. In contrast, outbreaks among school-aged children involve highly vaccinated populations. In some large school-aged outbreaks, over 95 percent of cases have occurred in

Measles, 1993-1999

Year	Cases
1993	312
1994	963
1995	309
1996	512
1997	138
1998	100
1999	86

Measles 1993-1999

- Less than 500 cases per year
- No predominant age group
- Interruption of transmission in the United States
- Most cases due to importation

Measles
Outbreaks

Outbreaks categorized by predominant age group affected

Preschool-aged	• Predominant age group <5 years • Most cases unvaccinated • Relatively large number of cases • Inner-city minority populations affected
School-aged	• Predominant age group 5-18 years • Most cases vaccinated • Relatively small number of cases • Junior and senior high schools

Measles Outbreaks, United States, 1985-1993

Location	Year(s)	No. Cases	Type
Los Angeles, CA	1987-92	12,226	Preschool
Chicago, IL	1989-90	3,100	Preschool
New York, NY	1990-92	3,065	Preschool
Dallas, TX	1989-90	2,300	Preschool
Houston, TX	1988-89	1,700	Preschool
Philadelphia, PA	1990-91	1,500	Preschool
New York, NY	1986-87	1,400	Preschool
Milwaukee, WI	1989-90	1,300	Preschool
San Diego, CA	1988-90	1,300	Preschool
Fresno, CA	1989-90	1,300	Preschool

persons with histories of vaccination on or after their first birthday (*i.e.*, because of vaccine failure). From 1985 through 1988, the majority of outbreaks occurred in highly vaccinated school-aged populations. An annual median of 47 school-aged outbreaks occurred, six of which involved >100 persons. These outbreaks accounted for a median of 51% of all reported measles cases. Outbreaks among preschool-aged children accounted for an annual median of 20% of reported cases during this time.

In 1989-1991, both the number and size of outbreaks increased, and preschool-type outbreaks became more prominent. Over 200 outbreaks were reported each year, several of which included more than 1000 cases each. These large outbreaks all involved predominately preschool-aged children. Large preschool outbreaks occurred in several inner city areas, including Los Angeles, Houston, Milwaukee, Chicago, Dallas, New York City, and Philadelphia. In these outbreaks, the majority of cases occurred among unvaccinated black and Hispanic children. In 1989-1991, outbreaks among school-aged children continued to occur, but accounted for a relatively small proportion of cases.

Since 1993, the largest outbreaks of measles have occurred in populations that refuse vaccination, including communities in Utah and Nevada, and Christian Scientist schools in Missouri and Illinois. Small outbreaks were reported in unvaccinated preschool populations, vaccinated school populations, college students, and adult communities, but these outbreaks were much smaller than those reported during 1989-1991. No large preschool-type outbreak has been reported since 1992.

Measles
Clinical Case Definition

- **Generalized rash lasting ≥3 days**
  AND
- **Temperature ≥38.3 C (101 F)**
  AND
- **Cough, or coryza, or conjunctivitis**

Classification of Measles Cases

Clinical classification of measles cases

A **suspect case** is a person with a febrile illness accompanied by a generalized maculopapular rash.

A **probable case** meets the **measles case definition** of generalized maculopapular rash lasting 3 days, with fever >38.3°C (101°F); and cough, or coryza, or conjunctivitis and has no or noncontributory serologic or virologic testing, and is not epidemiologically linked to a confirmed case.

A **confirmed case** meets the case definition, and is epidemiologically linked to another confirmed or probable case; or is laboratory confirmed. A laboratory confirmed case does not need to meet the clinical case definition.

Only confirmed cases should be reported to *Morbidity and Mortality Weekly Report (MMWR)*, but confirmed and probable cases should be reported as soon as possible to local or state health department.

Epidemiologic classification

An **imported case** has its source outside the country or state, rash onset occurs within 21 days after entering the country, and illness cannot be linked to local transmission.

An **indigenous case** is any case that cannot be proved to be imported. Subclasses of indigenous cases exist; see CDC Manual for Surveillance of Vaccine-Preventable Diseases for more information.

Measles Vaccine

Measles virus was first isolated by John Enders in 1954. The first measles vaccines were licensed in 1963. In that year, both an inactivated ("killed") and a live attenuated vaccine (Edmonston B strain) were licensed for use in the United States. The inactivated vaccine was withdrawn in 1967 because it did not protect against measles virus infection. Furthermore, recipients of inactivated measles vaccine frequently developed a unique syndrome, atypical measles, if infected with wild-type measles virus (see Atypical Measles, above). The original Edmonston B vaccine was withdrawn in 1975, because of a relatively high frequency of fever and rash in recipients. A live, further attenuated vaccine (Schwarz strain) was first introduced in 1965, but also is no longer used in the United States. Another live, further attenuated strain vaccine (Moraten strain) was licensed in 1968. These further attenuated vaccines caused fewer reactions than the original Edmonston B vaccine.

Measles Vaccine

1954	Measles virus isolated by Enders
1963	Licensure of attenuated live and killed vaccines
1965	Licensure of live further attenuate vaccine (Schwarz)
1967	Killed vaccine withdrawn
1968	Licensure of live further attenuated vaccine (Moraten)
1971	Licensure of combined measles-mumps-rubella vaccine
1989	Two-dose schedule

Characteristics

The only measles virus vaccine now available in the United States is a live, more attenuated Enders-Edmonston strain (formerly called "Moraten"). The vaccine is available as a single antigen preparation,

Measles Vaccine

• Composition	Live virus (Edmonston-Enders strain)
• Efficacy	95% (Range, 90%-98%)
• Duration of Immunity	Lifelong
• Schedule	2 Doses

Should be administered with mumps and rubella as MMR

combined with rubella vaccine, or combined with mumps and rubella vaccines. The ACIP recommends that combined measles-mumps-rubella vaccine (MMR) be used when any of the individual components is indicated.

MMR Vaccine Failure

- **2%-5% of recipients do not respond to the first dose**
- **Caused by antibody, damaged vaccine, record errors, other?**
- **Outbreaks may occur among nonresponders**

Measles vaccine is prepared in chick embryo fibroblast tissue culture. MMR is supplied as a lyophylized (freeze-dried) powder and is reconstituted with sterile, preservative-free water. The vaccine contains a small about of human albumin, neomycin, sorbitol, and gelatin.

Immunogenicity and vaccine efficacy

Measles vaccine produces an inapparent or mild, noncommunicable infection. Measles antibodies develop in approximately 95% of children vaccinated at 12 months of age and 98% of children vaccinated at 15 months of age. Approximately 2%-5% of children who receive only one dose of MMR vaccine fail to respond to it (*i.e.*, primary vaccine failure). MMR vaccine failure may occur because of passive antibody in the vaccine recipient, damaged vaccine, incorrect records, and possibly other reasons. Most children who fail to respond to the first dose will respond to a second dose. Studies indicate that more than 99% of persons who receive two doses of measles vaccine (with the first dose administered no earlier than the first birthday) develop serologic evidence of measles immunity.

Indications for Measles (MMR) Vaccine

- **All infants ≥12 months of age**
- **Susceptible adolescents and adults without documented evidence of immunity**

Although the titer of vaccine-induced antibodies is lower than that following natural disease, both serologic and epidemiologic evidence indicate that vaccine-induced immunity appears to be long-term and probably life-long in most individuals. Most vaccinated persons who appear to lose antibody show an anamnestic immune response upon revaccination indicating that they are probably still immune. Although revaccination can increase antibody titer in some persons, available data indicate that the increased titer may not be sustained. Some studies indicate that secondary vaccine failure (waning immunity) may occur after successful vaccination, but this appears to occur very rarely and to only play a minor role in measles transmission and outbreaks.

Measles Mumps Rubella Vaccine

- **12 months is the *recommended* and *minimum* age for MMR**
- **MMR given before 12 months should not be counted as a valid dose**
- **Revaccinate at ≥12 months of age**

Vaccination Schedule and Use

Two doses of measles vaccine, as combination MMR, separated by at least 4 weeks, are routinely recommended for all children. All persons born in or after 1957 should have documentation of at least one dose of MMR or other evidence of measles immunity (see below). Certain adolescents and adults should receive 2 doses of MMR.

The first dose of MMR should be given on or after the first birthday. Any dose of measles-containing vaccine given before 12 months of age should not be counted as part of the series. Children vaccinated with measles-containing vaccine before 12 months of age - even one day early - should be revaccinated with two doses of MMR vaccine, the first of which should be administered when the child is at least 12 months of age.

A second dose of MMR is recommended to produce immunity in those who failed to respond to the first dose. The second dose of MMR vaccine should routinely be given at age 4-6 years, before a child enters kindergarten or first grade. The preadolescent health visit at age 11-12 years can serve as a catch-up opportunity to verify vaccination status and administer MMR vaccine to those children who have not yet received two doses of MMR.

The second dose of MMR may be administered as soon as one month (*i.e.*, minimum of 28 days) after the first dose. Children who have already received two doses of MMR vaccine at least 4 weeks apart, with the first dose administered no earlier than the first birthday, do not need an additional dose when they enter school. Children without documentation of adequate vaccination against measles, rubella, and mumps or other acceptable evidence of immunity to these diseases when they enter school should be admitted after receipt of the first dose of MMR. A second dose should be administered as soon as possible, but no less than 4 weeks after the first dose.

Second Dose of Measles Vaccine

- **Intended to produce measles immunity in persons who failed to respond to the first dose (primary vaccine failure)**
- **May boost antibody titers in some persons**

Second Dose Recommendations

- **First dose of MMR at 12-15 months**
- **Second dose of MMR at 4-6 years**
- **Second dose *may* be given anytime >4 weeks after the first dose**

Vaccination of adults

Adults born in 1957 or later who do not have a medical contraindication should receive at least one dose of MMR vaccine unless they have documentation of vaccination with at least one dose of measles-, rubella-, and mumps-containing vaccine or other acceptable evidence of immunity to these three diseases. With the exception of women who might become pregnant (see rubella, Chapter 11) and persons who work in medical facilities, birth before 1957 generally can be considered acceptable evidence of immunity to measles, rubella, and mumps. Although not specifically recommended for most persons born before 1957, such adults, including those who may be at increased risk of acquiring severe measles, can receive two doses of MMR provided they are administered no less than 1 month (*i.e.* minimum of 28 days) apart and are not otherwise contraindicated.

Adults at Increased Risk of Measles

- **College students**
- **International travelers**
- **Health-care personnel**

Certain groups of adults may be at increased risk for exposure to measles and should receive special consideration for vaccination. These persons include persons attending colleges and other post-high school educational institutions, persons working in medical facilities, and international travelers.

Colleges and other post-high school educational institutions are potential high-risk areas for measles, rubella, and mumps transmission because of large concentrations of susceptible persons. Prematriculation vaccination requirements for measles immunity have been shown to significantly decrease the risk of measles outbreaks on college campuses where they are implemented and enforced. **Colleges, universities, technical and vocational schools, and other institutions for post-high school education should require documentation of two doses of MMR vaccine** or other acceptable evidence of measles, rubella, and mumps immunity before entry.

Students who have no documentation of live measles, rubella, or mumps vaccination or other acceptable evidence of measles, rubella, and mumps immunity at the time of enrollment should be admitted to classes only after receiving the first dose of MMR. A second dose of MMR should be administered no less than 4 weeks (*i.e.*, minimum of 28 days) later. Students with evidence of prior receipt of only one dose of MMR or other measles-containing vaccine on or after their first birthday should receive a second dose of MMR, provided at least one month has elapsed since their previous dose.

Measles Immunity in Health Care Personnel

<u>All</u> persons who work in medical facilities should be immune to measles

Persons who work in **medical facilities** are at higher risk for acquiring measles than the general population. Between 1985 and 1991, at least 795 measles cases occurred in adult health care workers, including nurses, physicians, laboratory and radiology technicians, clerks, assistants and students. An overall decline in measles incidence occurred after the 1989-91 measles resurgence with a total of 36 cases during 1993-96 occurring among persons working in medical facilities. However, transmission in a medical facility occurred in 15 of the 75 measles outbreaks reported during 1993-1996.

All persons who work within medical facilities should have evidence of immunity to measles and rubella. Because any health care worker (*i.e.*, medical or non-medical, paid or volunteer, full time or part time, student or non-student, with or without patient-care responsibilities) who is measles or rubella susceptible can contract and transmit these diseases, all medical facilities (*i.e.*, inpatient and outpatient, private and public) should ensure measles and rubella immunity among those who

work within their facilities (a possible exception might be a facility that treats only elderly patients considered at low risk for measles and rubella and their complications).

Adequate vaccination for measles and rubella for health care workers born during or after 1957 consists of two doses of a live measles-containing vaccine and at least one dose of a live rubella-containing vaccine. Health care workers needing a second dose of measles-containing vaccine should be revaccinated at least 4 weeks after their first dose.

Although birth before 1957 is generally considered acceptable evidence of measles and rubella immunity, medical facilities should consider recommending a dose of MMR vaccine to unvaccinated workers born before 1957 who do not have a history of prior measles disease or laboratory evidence of measles immunity, and those without laboratory evidence of rubella immunity.

Serologic screening need not be done before vaccinating for measles and rubella unless the medical facility considers it cost-effective. Serologic testing is appropriate only if tracking systems are used to ensure that tested persons who are identified as susceptible are subsequently vaccinated in a timely manner. Serologic testing for immunity to measles and rubella is not necessary for persons documented to be appropriately vaccinated or who have other acceptable evidence of immunity. If the return and timely vaccination of those screened cannot be assured, serologic testing before vaccination should not be done.

Measles Vaccine
Indications for Revaccination

- **Vaccinated before the first birthday**
- **Vaccinated with killed measles vaccine (KMV)**
- **Vaccinated with KMV followed by live vaccine within 3 months**
- **Vaccinated prior to 1968 with an unknown type of vaccine**
- **Vaccinated with IG in addition to a further attenuated strain or vaccine of unknown type**

Persons who travel outside of the United States are at increased risk of exposure to measles. Measles is endemic or epidemic in many countries throughout the world. Although proof of immunization is not required for entry into the United States, persons traveling or living abroad should have evidence of measles immunity. Adequate vaccination of persons who travel outside the United States is two doses of MMR.

Revaccination is recommended for certain persons. The following groups of persons should be considered unvaccinated and should receive at least one dose of measles vaccine. Those (1) vaccinated before the first

birthday, (2) vaccinated with killed measles vaccine (KMV), (3) vaccinated with KMV followed by live vaccine less than 4 months after the last dose of KMV, (4) vaccinated prior to 1968 with an unknown type of vaccine (the vaccine may have been KMV), (5) or vaccinated with IG in addition to a further attenuated strain or vaccine of unknown type (revaccination not necessary if IG was given with Edmonston B vaccine).

Post-exposure prophylaxis

Live measles vaccine provides permanent protection and may prevent disease if given within 72 hours of exposure. **Immune globulin (IG)** may prevent or modify disease and provide temporary protection if given within 6 days of exposure. The dose is 0.25 ml/kg body weight, with a maximum of 15 ml intramuscularly. The recommended dose of IG for immunocompromised persons is 0.5ml/kg of body weight (maximum 15 ml) intramuscularly. IG may be especially indicated for susceptible household contacts of measles patients, particularly contacts <1 year of age (for whom the risk of complications is highest). If the child is then 12 months of age or older, live measles vaccine should be given about 5 months later when the passive measles antibodies have disappeared. IG should not be used to control measles outbreaks.

Measles immunity

Measles Immunity

- **Born before 1957**
- **Documentation of physician-diagnosed measles**
- **Serologic evidence of immunity to measles**
- **Documentation of receipt of measles-containing vaccine**

Persons generally can be considered immune to measles if they 1) were born before 1957, 2) have documentation of physician-diagnosed measles, 3) have laboratory evidence of immunity to measles, or 4) have documentation of adequate vaccination. Criteria for adequate vaccination currently vary depending on state and local vaccination policy. In general, adequate vaccination for preschool-aged children (12 months of age and older) is one dose of MMR. For school- and college-age children, adequate vaccination is either one or two doses of MMR, depending on the vaccination requirements of the state and/or facility.

Persons working in medical settings are at higher risk of measles than the general population. As a result, adequate vaccination for persons born during or after 1957 who work in medical facilities consists of 2 doses of MMR or other live measles-containing vaccine separated by at least 4 weeks with the first dose administered no earlier than the first birthday. Although birth before 1957 is generally considered acceptable evidence of measles immunity, measles has occurred in some unvaccinated persons born before 1957. Medical facilities should consider recommending a dose of MMR for unvaccinated workers born before 1957 who

lack a history of measles disease or laboratory evidence of measles immunity.

Only doses of vaccine with written documentation of the date of receipt should be accepted as valid. Self-reported doses or a parental history of vaccination, by itself, is not considered adequate documentation. A health care worker should not provide an immunization record for a patient unless that health care worker has administered the vaccine or has seen a record that documents vaccination. Persons who lack adequate documentation of vaccination or other acceptable evidence of immunity should be vaccinated. Vaccination status and receipt of all vaccinations should be documented in the patient's permanent medical record.

Adverse Reactions Following Vaccination

Adverse reactions following measles vaccine (except allergic reactions) represent replication of measles vaccine virus with subsequent mild illness. These events occur 5-12 days postvaccination and only occur in persons who are susceptible to infection. There is no evidence of increased risk of adverse reactions following MMR vaccination in persons who are already immune to the diseases.

MMR Adverse Reactions

• Fever	5%-15%
• Rash	5%
• Joint symptons	25%
• Thrombocytopenia	<1/30,000 doses
• Parotitis	rare
• Deafness	rare
• Encephalopathy	<1/1,000,000 doses

Fever is the most common adverse reaction following MMR vaccination. Although measles, rubella, and mumps vaccines may cause fever after vaccination, the measles component of MMR vaccine is most often associated with this adverse event. After MMR vaccination, 5%-15% of susceptible persons develop a temperature of >103^{O} F (>39.4^{O}C) usually occurring 7-12 days after vaccination and generally lasting 1-2 days. Most persons with fever are otherwise asymptomatic.

Measles- and rubella-containing vaccines, including MMR, may cause a transient **rash**. Rashes, usually appearing 7-10 days after MMR or measles vaccination, have been reported in approximately 5% of vaccinees.

MMR vaccine may rarely cause **thrombocytopenia** (low platelet count) within the 2 months after vaccination. Estimates of the frequency of clinically apparent thrombocytopenia from Europe are 1 case per 30,000 to 40,000 vaccinated susceptible persons, with a temporal clustering of cases occurring 2 to 3 weeks after vaccination. The clinical course of these cases was usually transient and benign, although hemorrhage occurred rarely. The risk for thrombocytopenia during rubella or measles infection is much greater than the risk after vaccination. Based on case reports, the risk for MMR-associated thrombocytopenia may be higher for persons who have previously had immune thrombocytopenic purpura, particularly for those who had

thrombocytopenic purpura after an earlier dose of MMR vaccine.

Transient **lymphadenopathy** sometimes occurs following receipt of MMR or other rubella-containing vaccine and **parotitis** has been reported rarely following receipt of MMR or other mumps-containing vaccine.

Arthralgias and other **joint symptoms** are reported in up to 25% of susceptible adult women given MMR vaccine. This adverse event is associated with the rubella component (see Rubella, Chapter 11, for more details).

Allergic reactions following the administration of MMR or any of its component vaccines are rare. Most of these reactions are minor and consist of a wheal and flare or urticaria at the injection site. Immediate, anaphylactic reactions to MMR or its component vaccines are extremely rare. Allergic reactions including rash, pruritus, and purpura have been temporally associated with mumps vaccination, but are uncommon and usually mild and of brief duration.

To date there is no convincing evidence that any vaccine can cause autism or any kind of behavioral disorder. A suspected link between MMR vaccine and autism has been suggested by some parents of children with autism. Typically, symptoms of autism are first noted by parents as their child begins to have difficulty with delays in speaking after age one. MMR vaccine is first given to children at 12 to 15 months of age. Therefore autism cases with an apparent onset within a few weeks after MMR vaccination may simply be an expected but unrelated chance occurrence.

Contraindications and Precautions to Vaccination

MMR Vaccine
Contraindications and Precautions

- **Severe allergic reaction to prior dose or vaccine component**
- **Pregnancy**
- **Immunosuppression**
- **Moderate or severe acute illness**
- **Recent blood product**

Persons who have experienced a severe allergic reaction (*i.e.*, hives, swelling of the mouth or throat, difficulty breathing, hypotension, shock) following a prior dose of measles vaccine or to a vaccine component (*e.g.*, gelatin, neomycin), should generally not be vaccinated with MMR.

In the past, persons with a history of anaphylactic reactions following egg ingestion were considered to be at increased risk of serious reactions after receipt of measles- or mumps-containing vaccines, which are produced in chick embryo fibroblasts. However, recent

data suggest that anaphylactic reactions to measles- and mumps-containing vaccines are not associated with hypersensitivity to egg antigens, but to other components of the vaccines (such as gelatin). The risk for serious allergic reactions following receipt of these vaccines by egg-allergic persons is extremely low and skin-testing with vaccine is not predictive of allergic reaction to vaccination. Therefore, MMR may be administered to egg-allergic children without prior routine skin testing or the use of special protocols.

Measles and Mumps Vaccines and Egg Allergy

- **Measles and mumps viruses grown in chick embryo fibroblast culture**
- **Studies have demonstrated safety of MMR in egg allergic children**
- **Vaccinate without testing**

MMR vaccine does not contain penicillin. A history of penicillin allergy is not a contraindication to vaccination with MMR or any other U.S. vaccine.

Women known to be pregnant should not receive measles vaccine. Pregnancy should be avoided for 1 month following receipt of measles vaccine and 3 months following MMR vaccine. Close contact with pregnant women is **NOT** a contraindication to MMR vaccination of the contact. Breastfeeding is **NOT** a contraindication to vaccination of either the woman or the breastfeeding child.

Replication of vaccine viruses can be prolonged in persons who are **immunosuppressed or immunodeficient.** Severe immunosuppression can be due to a variety of conditions, including congenital immunodeficiency, HIV infection, leukemia, lymphoma, generalized malignancy, or therapy with alkylating agents, antimetabolites, radiation, or large doses of corticosteroids. Evidence based on case reports has linked measles vaccine virus infection to subsequent death in six severely immunocompromised persons. For this reason, **patients who are severely immunocompromised for any reason should not be given MMR vaccine**. Healthy susceptible close contacts of severely immunocompromised persons may be vaccinated.

In general, persons receiving **large daily doses of corticosteroids** (>2 mg/kg per day or >20 mg per day of prednisone) for 14 days or more should not receive MMR vaccine because of concern about vaccine safety. MMR and its component vaccines should be avoided for at least one month after cessation of high dose therapy.

Persons receiving low dose or short course (<14 days) therapy, alternate-day treatment, maintenance physiologic doses, or topical, aerosol, intra-articular, bursal, or tendon injections may be vaccinated. Although persons receiving high doses of systemic corticosteroids daily or on alternate days during an interval of less than

14 days generally can receive MMR or its component vaccines immediately after cessation of treatment, some experts prefer waiting until two weeks after completion of therapy.

Patients with leukemia in remission who have not received chemotherapy for at least 3 months may receive MMR or its component vaccines.

Measles disease may be severe in persons with HIV infection. Available data indicate that vaccination with MMR has not been associated with severe or unusual adverse events in HIV-infected persons without evidence of severe immunosuppression, although antibody responses have been variable. MMR vaccine is recommended for all asymptomatic HIV-infected persons, and should be considered for symptomatic persons who are not severely immunosuppressed. Asymptomatic children do not need to be evaluated and tested for HIV infection before MMR or other measles-containing vaccines are administered. A theoretical risk of an increase (probably transient) in HIV viral load following MMR vaccination exists because such an effect has been observed with other vaccines. The clinical significance of such an increase is not known.

Measles Vaccine and HIV Infection

- **MMR recommended for persons with asymptomatic HIV infection**
- **NOT recommended for those with evidence of severe immuno-suppression**
- **Prevaccination HIV testing not recommended**

MMR and other measles-containing vaccines are not recommended for HIV-infected persons with evidence of severe immunosuppression (see table), primarily because of the report of a case of measles pneumonitis in a measles vaccinee who had an advanced case of AIDS.

Age-specific CD4+ T-lymphocyte count and percent of total lymphocytes as criteria for severe immunosuppression in HIV-infected persons.

Criteria	age <12 months	age 1-5 years	age 6-12 years	age ≥13 years
Total CD4+ T-lymphocytes	<750 per µL	<500 per µL	<200 per µL	<200 per µL
OR	***OR***	***OR***	***OR***	***OR***
CD4+ T-lymphocytes (as % of total lymphocytes)	<15 %	<15 %	<15 %	<14 %

Persons with **moderate to severe acute illness** should not be vaccinated until the illness has resolved. This precaution is intended to prevent complicating the management of an ill patient with a potential vaccine adverse event, such as fever. Minor illness (*e.g.*, otitis media, mild upper respiratory infections), concurrent antibiotic therapy, and exposure or recovery from other illness are not contraindications to measles vaccination. One recent study suggested that seroconversion to

measles vaccine was reduced in children with upper respiratory infections. However, multiple previous and subsequent studies have not confirmed this finding.

Receipt of antibody-containing blood products (*e.g.*, immune globulin, whole blood or packed red blood cells, intravenous immune globulin) may interfere with seroconversion to measles vaccine. The length of time that such passively acquired antibody persists depends on the concentration and quantity of blood product received. For instance, vaccination is recommended to be delayed for 3 months following receipt of immune globulin for prophylaxis of hepatitis A, but a 7-11 month delay is recommended following administration of intravenous immune globulin, depending on the dose. For more information, see Chapter 2 of this book and Measles Vaccine and Antibody Table in Appendix 4.

Persons who have a history of thrombocytopenic purpura or **thrombocytopenia** may be at increased risk for developing clinically significant thrombocytopenia after MMR vaccination. No deaths have been reported as a direct consequence of vaccine-induced thrombocytopenia. The decision to vaccinate with MMR depends on the benefits of immunity to measles, mumps, and rubella and the risks for recurrence or exacerbation of thrombocytopenia after vaccination or during natural infection with measles or rubella. The benefits of immunization are usually greater than the potential risks, and administration of MMR vaccine is justified, because of the even greater risk for thrombocytopenia after measles or rubella disease. However, deferring a subsequent dose of MMR vaccine may be prudent if the previous episode of thrombocytopenia occurred within 6 weeks after the previous dose of the vaccine. Serologic evidence of measles immunity in such persons may be sought in lieu of MMR vaccination.

Tuberculin testing (PPD) is not a prerequisite for vaccination with MMR or other measles-containing vaccine. PPD testing has no effect on the response to MMR vaccination. However, measles vaccine (and possibly mumps, rubella, and varicella vaccines) may suppress the response to PPD in a person infected with *Mycobacterium tuberculosis*. To minimize the risk of a false-negative interpretation, PPD testing should be delayed for 4-6 weeks after MMR vaccination. If PPD testing is needed, it should be done prior to MMR vaccination. It is also acceptable to apply the PPD and administer MMR simultaneously, since the mild immunosuppressive effect of the vaccine will not occur for several days after vaccination.

PPD and Measles Vaccine

- **Apply PPD first - give MMR when skin test read**
- **Apply PPD at same time as MMR**
- **Delay PPD 4-6 weeks if MMR given first**

Vaccine Storage and Handling

Measles vaccine and MMR must be shipped with refrigerant to maintain 10°C (50°F) or less at all times. Vaccine must be refrigerated immediately on arrival and protected from light at all times. The vaccine must be stored at refrigerator temperature (2°-8°C [35°-46°F]), but may be frozen. Diluent may be stored at refrigerator temperature or at room temperature.

After reconstitution, measles and MMR vaccines must be stored at refrigerator temperature and protected from light. Reconstituted vaccine should be used immediately. If reconstituted vaccine is not used within 8 hours it must be discarded.

Summary - Measles

- **<1000 cases per year**
- **Most cases due to importation**
- **Adults account for almost a third of remaining cases**
- **2 dose recommendation**

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MEASLES VIRUS ISOLATION

Background

The availability of a sensitive cell line (B95a) for isolation of measles virus from clinical specimens and the establishment of automated DNA sequencing techniques have allowed for rapid genetic characterization of a large number of wild-type strains of measles virus. This database of sequence information now makes it possible to use molecular epidemiological techniques to identify the source of wild-type viruses and to rapidly differentiate between wild-type and vaccine strains. As we progress toward elimination of measles in the U.S., it will be critical to examine virus isolates from as many outbreaks as possible in order to identify the source of the virus.

Virus isolation and genetic characterization can take several months to complete. Therefore, **diagnosis of measles should always be based on detection of measles-specific IgM in serum**. The IgM-capture EIA test can be completed in one day. Specimens for virus isolation can and should be taken **at the same time** that serum is obtained, since a delay in collection will reduce the chance of isolating the virus. However, **urine or nasopharyngeal or throat swab specimens should not be substituted for serum specimens for measles diagnosis**.

We are currently testing the possibility of using saliva in addition to serum to diagnose measles using the IgM-capture EIA. During a measles outbreak, if you believe that you can collect saliva **in addition to serum** to help us to test this method, please contact us and we will send you saliva collection kits. After collection, we would request that you can send us an aliquot of serum with the saliva.

Detailed protocols are available for the collection and processing of specimens for measles virus isolation. Please contact the National Immunization Program, or Dr. William Bellini, in the Measles Virus Section (404-639-3512) for the protocol.

4. Keep all specimens on wet ice or at 4° C and ship as soon as possible on wet ice (see address below).

If immediate, cold shipment (within 48 hr.) cannot be arranged or is not convenient, nasal wash specimens can be centrifuged at 2500 x g for 15 minutes at 4° C and the pellet resuspended in 1 ml of tissue culture medium. If possible, the supernatant can be saved in a separate tube. The samples should be frozen and shipped at -70° C (dry ice). If centrifugation is not available the whole specimen can be frozen (preferably at -70° C) and shipped on dry ice.

Nose and throat swabs can be removed from the transport medium after allowing some time for elution of virus. The specimen can then be frozen at -70 C and shipped on dry ice.

B. Urine specimens

We prefer urine to be collected in the first week after rash onset, though we will accept urines up to 14 days after rash onset. Virus has been isolated from the urine for up to one week after the onset of rash. We will also accept urine from close contacts of measles cases (e.g., household contacts) if they are collected 14 days after the rash onset of the measles case. First morning voided specimens are ideal, but any urine collection is adequate. Collect up to 50-100 ml of urine in 50 ml centrifuge tubes or a urine specimen container. Centrifuge at 2500 x g for 15 minutes at 4° C to pellet the sediment. Resuspend the sediment in 2-3 ml of VTM (above) or any cell culture medium (DMEM, EMEM, RPMI plus antibiotics) and ship. Preferably specimens that have been centrifuged and resuspended should be frozen at -70° C and shipped on dry ice. If dry ice is not available, however, they can be stored at 4° C and shipped on wet ice.

If centrifugation is not available, do not freeze the urine sample. The entire urine specimen should be stored at 4° C, and shipped to the lab on wet ice.

C. Virus Isolation:

The marmoset lymphocyte cell line B95a is Epstein-Barr virus transformed and should be handled as an infectious cell line capable of yielding Epstein-Barr virus (Kobune et al., 1990, J Virol. 64:700-705). This cell line is useful in measles virus isolation with syncytial and giant-cell cytopathogenic effect (CPE) sometimes visible as early as 48 hours after inoculation. Isolation-attempt cultures should be followed for 7-8 days with subsequent passages 2-3 times before ruling out possible isolation of measles virus. B95-8 cells are available from the American Type Culture Collection (# CRL 1612). When cultured in Dulbecco's Modified Minimum Essential Medium (DMEM), these cells will adhere to the surface of the culture vessel and the adherent cells are referred to as B95a.

The cells grow gently attached to the bottom surface of the flask when grown in DMEM supplemented with 100 units/ml penicillin, 100 mg/ml streptomycin, 0.25 ug/ml amphotericin (fungizone), and fetal bovine serum. Cell growth is sustained by adding 5-10% fetal bovine serum (FBS). FBS is used at a 2% concentration for cell maintenance during viral isolation.

The B95a cells can be passaged by briefly treating the cell monolayers with 0.05% trypsin-EDTA to release cells from cell culture flask surface. Be careful not to over trypsinize. Neutralize trypsin by adding DMEM containing FBS. Usually the cells from a single monolayer culture can be split 1:3. One will notice that more cells tend to become "floaters", growing in clumps suspended in the medium as the cell density increases. These cells are viable and can be passaged by gently pipetting to break up the clumps then replating to a lower cell density.

Cells can be transported in a T-75 or T-25 tissue culture flask with additional medium added to help keep cells attached. Upon arrival look at the cell sheet. If many cells are free-floating, a light spin of the medium will recover cells which can be added back to the flask (or to another flask for passage). Add 30-35 mls of the medium back to the flask for maintenance. Grow cells in a moist $C0_2$ incubator at 37° C. Cell stocks can be frozen using standard cryoprotection medium (20% FBS, 10% DMSO).

Inoculation.
1. Passage cells, split into T-25 flasks @ 1:3 or 1:4 and incubate 24-48 hours.

2. Decant medium, add 1-1.5 ml DMEM w/2X antibiotics, add 0.1 to 1.0 ml of clinical specimen depending on concentration.

3. Incubate at 37 C. for 1 hour.

4. Decant medium into bleach water, replace medium with DMEM containing 2% FBS and 2X antibiotics.

5. Change medium every 3-4 days and passage cells by splitting @ 1:3 every 7-9 days. Check for CPE daily.

6. Attempt at least three passages before discontinuing isolation protocol. Do not discard remaining clinical specimens as they may still be used for PCR analysis.

7. If CPE is visible, continue to feed the cells until the CPE becomes extensive. It may be necessary to passage the cells one time to allow the CPE to progress. When CPE is maximal, pellet cells and freeze at -70 C.

If successful virus isolation has been performed confirmation can be achieved by using an immunological assay such as fluorescent antibody or by PCR. Please remember to save some of the original clinical specimen. This material can be used for a second isolation attempt if problems occur with the first as well as provide a specimen for PCR analysis.

Alternatively, infected cells can be pelleted, resuspended in a small volume of medium and frozen at -70^{o} C before shipping on dry ice.

D. Shipping:
Viral isolates (not clinical specimens) arriving from overseas will require CDC and USDA Import Permits. Please call numbers below to obtain a permit.

If Federal Express is available, contact the CDC to arrange pre-paid transport. If not, CDC will be able to re-imburse for shipping costs.

Ship to:

Dr. William J. Bellini
Measles Virus Section, REVB, C-22
DASH Group #81
Centers for Disease Control and Prevention
1600 Clifton Rd.
Atlanta, GA 30333 USA
tel: 404-639-3512, 404-639-3308
fax: 404-639-4187
e-mail: wjb2@cdc.gov

Please FAX CDC with any questions or to arrange shipping.

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